Remarks

Upon entry of the foregoing amendments, claims 68-120 are pending in the application, with the independent claim being claim 68. Claim 114 has been amended to correct a typographical error in the dependency, and claims 112 and 113 have been amended to clarify the terms "v7.5/tk" and "vEL/tk," respectively. The amendments do not narrow the scope of the claims. Support for the amendments may be found at page 20, lines 10-28; page 42, line 19 to page 43, line 17; page 71, line 23 to page 72, line 2; and page 78, line 14 to page 79, line 19.

The specification has been amended to conform with the formal drawings submitted herewith and to correct obvious typographical errors. The title has been amended to better reflect the claimed subject matter.

It is believed that these changes introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Objections: Drawings

Figures 2, 3, and 9 were objected to as containing informalities. Applicant submits herewith formal drawings to correct the formalities. Accordingly, it is respectfully believed that the objection is now moot.

Objections: Abstract

The abstract was objected to for reciting the word "novel." Applicant has amended the abstract to cancel the offending word. Accordingly, it is respectfully believed that the objection is now moot.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 112 and 113 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for reciting the terms "v7.5/tk" and "vEL/tk," respectively. Applicant respectfully traverses.

Applicant believes that the terms "v7.5/tk" and "vEL/tk" are clear and definite to one of ordinary skill in the relevant art. Nevertheless, Applicant has amended claim 112 to recite a vaccinia virus genome comprising "a modified thymidine kinase (tk) gene which comprises a 7.5k promoter, a unique NotI restriction site, and a unique ApaI restriction site" rather than the phrase a "v7.5/tk" virus genome. Likewise, claim 113 has been amended to recite a vaccinia virus genome comprising "a modified thymidine kinase (tk) gene which comprises a synthetic early/late (E/L) promoter, a unique NotI restriction site, and a unique ApaI restriction site" rather than a "vEL/tk" virus genome. While not acquiescing to the rejection, Applicant believes amended claims 112 and 113 are clear and definite.

Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

Rejections Under 35 U.S.C. § 102

Claims 68-70 and 120 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Wolfel et al., U.S. Patent No. 5,530,096 (the '096 patent). (Paper No. 33, p. 5.) The Office Action states that the '096 patent teaches

> a method for selecting a nucleic acid molecule encoding a target epitope of CTL comprising contacting host cells with CTLs specific for said target epitope wherein said host cells comprise a vaccinia virus library of heterologous nucleic acid molecules encoding said target epitope in host cells . . . and recovering those host cells which have undergone a lytic event by measuring TNF Values.

(*Id.*) The Office Action also states that the '096 patent teaches "removing the vector from positive clones that have undergone a lytic event" and "isolating the vector from the host cells and transferring them to an expression vector in a host" and repeating the process. (*Id.*) Applicant respectfully disagrees with the rejection.

For a prior art reference to anticipate the claimed invention, it must disclose all of the elements of the claim. *Finnigan Corp. v. United States Int'l Trade Comm'n*, 180 F.3d 1354, 1365-66, 51 USPQ2d 1001, 1008 (Fed. Cir. 1999). In the present case, the Office Action failed to establish a prima facie case of anticipation.

Applicant asserts that the pending claims are novel over the method disclosed in the '096 patent. The method of claims 68-70 and 120 requires that a host cell expressing the target epitope undergoes a lytic event upon interaction with a specific cytotoxic T-lymphocyte. Recovery of those host cells harboring the desired recombinant vector is made by *collecting the actual host cells which have undergone a lytic event.* From these recovered cells, the nucleic acid molecule encoding the desired target epitope is isolated.

In the method taught in the '096 patent, recombinant plasmid DNAs were first isolated from hundreds of pools, each containing 200 bacterial transformants, and were used to transfect eukaryotic (COS-7) cells. '096 patent, col. 4, lines 40-55. These cells were then screened for the ability to stimulate the release of TNF from a specific CTL clone, as measured by adding supernatant to WEHI cells and measuring subsequent WEHI cell lysis. *Id.*, col. 5, lines 5-11. Neither the actual host cells carrying the desired plasmids nor the desired plasmids themselves were recovered from the transfected COS-7 cells. Instead, once a positively reacting pool was identified, individual plasmids from the earlier-transformed bacteria were used to transfect new eukaryotic cells, to repeat the transfection and screening process until a unique cDNA clone was identified. *Id.*, lines 21-46.

Thus, the method in the '096 patent is a *screening assay*, not a selection method. The screening assay of the '096 patent differs from the *selection method* of the captioned application in that the host cells are not recovered, or selected, from the assay based on their CTL reactivity. Applicant notes that the presently claimed selection method, as typified in claim 68, is far more efficient than the screening process described in the '096 patent, since it is not necessary to separately assay the many negative pools and clones. In other words, the claimed method may be carried out using only *one* "pool," which contains *all* the clones, both positive and negative. In contrast, in the '096 patent, *700* pools were separately assayed to identify the four pools containing positive clones. '096 patent, col. 4, lines 40-42, and col. 5, lines 13-14.

At a minimum, the '096 patent fails to teach or suggest collecting the actual host cells which react with CTL, as recited in claims 68 and 70, and fails to teach or suggest isolating

vector from the actual host cells which react with CTL, as recited in claims 69 and 70.[1] Therefore, the '096 patent fails to teach or suggest all the elements of claims 68-70. Because claim 120 depends from and therefore include all the elements of claim 68, the '096 patent also fails to teach or suggest all the elements of this claim.

Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

Rejections Under 35 U.S.C. § 103

Claims 68-111, 114-115, and 117-120 were rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Wolfel et al., U.S. Patent No. 5,530,096 (the '096 patent) in view of Paoletti et al., U.S. Patent No. 5,494,807 (the '807 patent). (Paper No. 33, p. 6.) As discussed above, the Office Action states that the '096 patent teaches

> a method for selecting a nucleic acid molecule encoding a target epitope of CTL comprising contacting host cells with CTLs specific for said target epitope wherein said host cells comprise a vaccinia virus library of heterologous nucleic acid molecules encoding said target epitope in host cells . . . and recovering those host cells which have undergone a lytic event by measuring TNF Values.

(*Id.*, p. 5.) The Office Action also states that the '096 patent teaches "removing the vector from positive clones that have undergone a lytic event" and "isolating the vector from the host cells and transferring them to an expression vector in a host" and repeating the process. (*Id.*)

Regarding the '807 patent, the Office Action states that it teaches "a vaccinia vector that expresses heterologous epitopes" and teaches that

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[1] Additionally, the '096 patent fails to teach or suggest the production of a library in *vaccinia virus*, contrary to the statement in the Office Action. (Paper No. 33, p. 5.)

> the vaccinia vector can be used for expressing a gene product in a cell cultured in vitro by introducing into the cell a modified recombinant virus having attenuated virulence and enhanced safety.

(*Id.*, pp. 6-7.) The Office Action concludes that "it would have been obvious to one of skill in the art to have substituted the vaccinia expression vector" of the '807 patent for the vectors of the '096 patent "in a method for identifying target epitopes" taught by the '096 patent and that there would have been an expectation of success. (*Id.*, p. 7.) Applicant respectfully disagrees with the rejection.

To establish a prima facie case of obviousness under 35 U.S.C. § 103, the Examiner must show that the prior art suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process, and that the invention could be attained with a reasonable expectation of success. See *In re Vaeck*, 20 U.S.P.Q.2d (BNA) 1438, 1442 (Fed. Cir. 1991). The present Office Action failed to carry this burden.

Applicant asserts that the pending claims are nonobvious over the '096 patent in combination with the '807 patent. As discussed above with respect to the rejection under 35 U.S.C. § 102, the '096 patent, at a minimum, fails to teach or suggest collecting the actual host cells that react with CTL, as recited in claim 68.

The '807 patent also fails to teach or suggest the method of claim 68. The '807 patent teaches the deletion from vaccinia virus of regions encoding known or potential virulence factors. '807 patent, *e.g.*, col. 8, lines 46-50; cols. 9-17 (examples 1-7); cols. 77-81 (example 17). The '807 patent also teaches the insertion of previously isolated genes into the vaccinia virus deletion constructs. *Id.*, *e.g.*, col. 17-77 (examples 8-16). The '807 patent does not

teach or suggest how to modify the method of the '096 patent to arrive at the method of claim 68, and therefore fails to cure the defects of the '096 patent.

Thus, the '096 patent, either alone or in combination with the '807 patent, fails to teach or suggest all the elements of claim 68. Since claims 69-111, 114-115, and 117-120 depend from and therefore include all the elements of claims 68, the '096 patent, alone or in combination with the '807 patent, also fails to teach or suggest all the elements of these claims. The pending claims are therefore nonobvious over the cited patents.

Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

In the Title:

The title has been amended as follows:

Methods for Selecting Polynucleotides Encoding T Cell Epitopes ~~T Cells Specific for Target Antigens and Vaccines Based Thereon~~

In the Substitute Specification:

The paragraph beginning on page 10, line 29 was amended as follows:

FIGs. 8A and 8B. Differential expression in tumor lines of differential display clone 90. RNase ~~Rnase~~ protection assay: 300 picograms of clone 90 antisense probe was hybridized with 5 micrograms total RNA prior to RNase digestion and analysis of protected fragments on 5% denaturing PAGE.

The paragraph beginning on page 11, line 21 was amended as follows:

FIGs. 11A and 11B. Southern Blot Analysis of Viral Genomes p7.5/tk (FIG. 11A) and pEL/tk (FIG. 11B). The viruses v7.5/tk and vEL/tk were used to infect a well of a 6 well dish of BSC-1 cells at high multiplicity of infection (moi) and after 48 hours the cells were harvested and the DNA was isolated using DNAzol (Gibco). The final DNA product was resuspended in 50 microliters of TE 8.0 and 2.5 microliters were digested with HindIII, HindIII and ApaI, or HindIII and Not I, electrophoresed through a 1.0% agarose gel, and transferred to Nytran (Schleicher and Schuell) using a Turboblotter (Schleicher and Schuell). The samples were probed with p7.k/tk (FIG 11A ~~a~~) or pEL/tk (FIG. 11B ~~b~~) labeled with ^{32}P using Random Primer DNA Labeling Kit (Bio-Rad) in QuickHyb (Stratagene). The lower portion of the figure denotes a map of the HindIII J fragment with the positions of the HindIII, NotI, and ApaI sites illustrated. The leftmost 0.5 kilobase fragment has electrophoresed off the bottom of the gel.

The paragraph beginning on page 62, line 11 was amended as follows:

In preliminary experiments, an average of three differentially displayed bands were identified for each pair of primers. With a total of 66 primer pairs generated from all possible combinations of 12 independent primers, approximately 200 gene fragments could be identified. In some cases multiple fragments may derive from the same gene. FIG. 7 shows the pattern of differential display fragments observed with one pair of arbitrary decamers, MR_1 (TAC AAC GAG G) (SEQ ID NO:11) and MR_5 (GGA CCA AGT C) (SEQ ID NO:13). A number of bands can be identified that are associated with all four tumors but not with the parental cells. This distribution is unrelated to the immunogenicity of the tumor cells, since only three of the four tumors are immunologically crossreactive. In contrast to the differentially expressed bands identified by RDA, which gave positive results on the Northern blots exposed for only a few hours, fragments identified by differential display did not give a signal on Northern blots even after several days. Differential expression of the differential display fragments can, however, be confirmed by RNase protection assays or by semi-quantitative PCR with sequence specific primers. An example is shown in FIGs. 8A and 8B the results of an RNase protection assay with clone 90 from differential display band 9. This sequence, which has no significant homology to entries in the GenBank database, is expressed in all four tumor lines but not in the parental B/c.N.

The paragraph beginning on page 76, line 5 was amended as follows:

One well of a 6 well dish of BSC-1 was infected with v7.5/tk or vEL/tk at high multiplicity of infection (moi) and after 48 hours the cells were harvested, pelleted by low speed centrifugation, rinsed with Phosphate-Buffered Saline (PBS), and the DNA was isolated using DNAzol (Gibco). The final DNA product was resuspended in 50 microliters of TE (10mM TrisHCl, pH 8.0~~-~~1 mM EDTA) and 2.5 microliters were digested with HindIII, HindIII and ApaI, or HindIII and NotI, electrophoresed through a 1.0% agarose gel, and transferred to Nytran (Schleicher and Schuell) using a Turboblotter (Schleicher and Schuell). The samples were probed with p7.5/tk (FIG. 11A~~a~~) or pEL/tk (FIG. 11B~~b~~) labeled with ^{32}P using Random Primer DNA Labeling Kit (Bio-Rad) in QuickHyb (Stratagene) and visualized on Kodak XAR film.

The paragraph beginning on page 80, line 27 was amended as follows:

The genomes for vEL/tk and v7.5/tk were analyzed by Southern blotting to confirm the location of the ApaI and NotI sites in the HindIII J fragment as shown in FIGs. 11A and 11B. The filters were hybridized to ^{32}P labeled HindIII J fragment derived from the p7.5/tk or pEL/tk. The genomes for v7.5/tk and vEL/tk have an ApaI site that does not appear in vNotI/tk (compare lanes 7 and 8 to lane 5 in each blot) whereas digestion with NotI and HindIII yield a set of fragments of equivalent size. The 0.5 kilobase HindIII/NotI or HindIII/ApaI fragment from the left hand side of HindIII J produced from NotI or ApaI digestion has electrophoresed off the bottom of the agarose gel.

In the Claims:

Claims 112-114 were amended as follows:

112. (once amended) The method of claim 103, wherein said vaccinia virus genome comprises a modified thymidine kinase (tk) gene which comprises a 7.5k promoter, a unique NotI restriction site, and a unique ApaI restriction site ~~isolated virus genome is a v7.5/tk virus genome~~.

113. (once amended) The method of claim 103, wherein said vaccinia virus genome comprises a modified thymidine kinase (tk) gene which comprises a synthetic early/late (E/L) promoter, a unique NotI restriction site, and a unique ApaI restriction site ~~isolated virus genome is a vEL/tk virus genome~~.

114. (once amended) The method of claim 103 ~~102~~, wherein the 5' and 3' flanking regions of said transfer plasmids are capable of homologous recombination with a vaccinia virus thymidine kinase gene.

In the Abstract:

The abstract on the last page of the original specification has been amended as follows:

The present invention relates to ~~novel~~ methods for the identification of antigens recognized by cytotoxic T cells (CTLs) and specific for human tumors, cancers, and infected cells, and the use of such antigens in immunogenic compositions or vaccines to induce regression of tumors, cancers, or infections in mammals, including humans. The invention encompasses methods for induction and isolation of cytotoxic T cells specific for human tumors, cancers and infected cells, and for improved selection of genes that encode the target antigens recognized by these specific T cells. The invention also relates to differential display methods that improve resolution of, and that reduce the frequency of false positives of DNA fragments that are differentially expressed in tumorous, cancerous, or infected tissues versus normal tissues. The invention further relates to the engineering of recombinant viruses as expression vectors for tumor, cancer, or infected cell-specific antigens.